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Chronic Toxicity of Chlordane, Trifluralin, and Pentachlorophenol to Sheepshead Minnows (Cyprinodon variegatus)

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16. ABSTRACT Sheepshead minnows (Cyprinodon variegatus) were exposed to three chemicals--chlordane, trifluralin, or pentachlorophenol--in flowing, natural seawater to determine acute and chronic (full life-cycle effects).

Mortality of parental fish exposed to mean measured chlordane concentrations ≥2.8 µg/ℓ was significantly greater than that of control fish. Hatch of juveniles from embryos of parental fish exposed to ≥0.8 µg/ℓ was significantly less than hatch of control juveniles. The estimated maximum acceptable toxicant concentration (MATC) was >0.5<0.8 µg/ℓ and the application factor (AF) limits, 0.04-0.06.

Exposure to mean measured trifluralin concentrations ≥9.6 µg/ℓ significantly decreased growth of parental fish. Fecundity of parental fish exposed to concentrations ≥4.8 µg/ℓ was significantly less than that of control fish. Survival and growth of second generation fish were significantly less than the control in concentrations ≥9.6 µg/ℓ. The estimated MATC was >1.3<4.8 µg/ℓ and the AF limits, 0.007-0.025.

Mortality of parental sheepshead minnows exposed to mean measured pentachlorophenol concentrations >88 µg/ℓ was significantly greater than mortality of control fish. The estimated MATC was >47<88 µg/ℓ and the AF limits, 0.11-0.20.

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CHRONIC TOXICITY OF CHLORDANE, TRIFLURALIN, AND PENTACHLOROPHENOL TO SHEEPSHEAD MINNOWS (Cyprinodon variegatus)

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FOREWORD

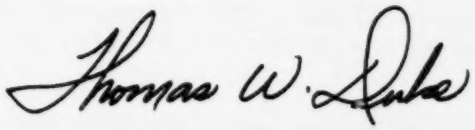
The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing concentration-response relationships for organisms and ecosystems under varying conditions is required. The Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

The effects of toxic organic pollutants on individual species and communities of organisms;

The effects of toxic organics on ecosystem processes and components;

The significance of chemical carcinogens in the estuarine and marine environments.

This report describes effects of three chemicals in full life-cycle tests with an estuarine fish, the sheepshead minnow. The data will be useful in establishing estuarine water quality criteria, and limiting effluents containing chlordane, trifluralin, or pentachlorophenol.



Thomas W. Duke
Director
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ABSTRACT

Sheepshead minnows (Cyprinodon variegatus) were exposed to each of three chemicals--chlordane, trifluralin, or pentachlorophenol--in flowing, natural seawater to determine acute and chronic (full life-cycle) effects. The calculated 96-hour LC50's and 95% confidence limits, based on measured concentrations, were: chlordane, 12.5 micrograms per liter (µg/ℓ), 3.4-45.9 µg/ℓ; trifluralin, 190 µg/ℓ, 128-282 µg/ℓ; and pentachlorophenol, 442 µg/ℓ, 308-635 µg/ℓ.

In a chronic test, sheepshead minnows were exposed to mean measured concentrations of chlordane (0.5-18.0 µg/ℓ) for 189 days. Exposure to concentrations ≥2.8 µg/ℓ caused significant ($P<0.5$) mortality of parental fish. Exposure to chlordane concentrations ≥0.8 µg/ℓ significantly reduced hatch of embryos spawned by parental fish and exposure to concentrations ≥1.7 µg/ℓ caused significant mortality of second generation fish. The estimated maximum acceptable toxicant concentration (MATC) of chlordane for sheepshead minnows was >0.5<0.8 µg/ℓ; the application factor (AF) limits were 0.04-0.06.

Sheepshead minnows were exposed to mean measured concentrations of trifluralin (1.3-34.1 µg/ℓ) for 166 days. Exposure to concentrations ≥17.7 µg/ℓ caused significant mortality of parental fish. Exposure to trifluralin concentrations ≥9.6 µg/ℓ significantly reduced growth of parental fish and exposure to concentrations ≥4.8 µg/ℓ significantly reduced fecundity of parental fish. Exposure to concentrations ≥9.6 µg/ℓ significantly reduced hatch of embryos spawned by parental fish, and survival and growth of second generation fish. The estimated MATC of trifluralin for sheepshead minnows was >1.3<4.8 µg/ℓ; the AF limits were 0.007-0.025.

Sheepshead minnows were exposed to mean measured concentrations of pentachlorophenol (18-389 µg/ℓ) for 151 days. Exposure to concentrations ≥88 µg/ℓ caused significant mortality of parental fish. Exposure to pentachlorophenol concentrations ≥195 µg/ℓ significantly reduced hatch of embryos spawned by parental fish and survival of second generation fish. The estimated MATC of pentachlorophenol for sheepshead minnows was >47<88 µg/ℓ; the AF limits were 0.11-0.20.

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SECTION I

INTRODUCTION

Methods for chronic (full life-cycle) toxicity tests with freshwater fishes are well established. In contrast, until recently little was known about the effects of toxicants on complete life cycles of saltwater fishes. The use of sheepshead minnows (Cyprinodon variegatus) for life-cycle tests was proposed by Schimmel and Hansen (1975) and a tentative method was described by Hansen and Schimmel (1975). Results of several partial and full life-cycle tests with sheepshead minnows were reported more recently (Hansen and Parrish, 1977; Hansen et al., 1977; and Parrish et al., 1977). Our studies were undertaken to gain information essential to the determination of marine water quality criteria. Laboratory toxicity tests were conducted, the results of which allowed estimation of "safe" and "unsafe" concentrations of chlordane, trifluralin, and pentachlorophenol for survival, growth, and reproduction of sheepshead minnows.

Chlordane is an organochlorine pesticide which has been widely used, especially for the control of soil pests (U.S. Department of Health, Education, and Welfare, 1969). Although chlordane is relatively insoluble in water (6-9 micrograms per liter; µg/ℓ) and does not migrate readily through soil (National Research Council of Canada, 1975), measurable concentrations of chlordane have been found in major U.S. river basins, in the Great Lakes, and in southeastern U.S. estuaries (Henderson et al., 1969; Henderson et al., 1971; and Bugg et al., 1967).

That technical chlordane is acutely toxic to nontarget organisms has been shown in tests with freshwater invertebrates and fishes (Cardwell et al., 1977) and saltwater invertebrates and fishes (Parrish et al., 1976). The waterflea, Daphnia magna, was the most sensitive freshwater organism; the 96-hour LC50 (concentration estimated to be lethal to 50% of the test organisms) was 28 µg/ℓ (with 95% confidence limits of 25-32 µg/ℓ). Cardwell et al. (op. cit.) also reported that freshwater fish were nearly as sensitive as Daphnia, with 96-hour LC50's (and 95% confidence limits) of 37 µg/ℓ (33-41 µg/ℓ) for fathead minnows (Pimephales promelas); 47 µg/ℓ (no confidence limits) for brook trout (Salvelinus fontinalis); and 59 µg/ℓ (50-71 µg/ℓ) for bluegill (Lepomis macrochirus). Parrish et al. (op. cit.) found one saltwater invertebrate to be much more acutely sensitive to chlordane than were the freshwater invertebrates or fishes. Two other

saltwater invertebrates and two fishes were slightly more sensitive than were the freshwater species. The 96-hour LC50 (and the 95% confidence limits) for pink shrimp (Penaeus duorarum) was 0.4 µg/ℓ (0.3-0.6 µg/ℓ); the 96-hour EC50 (concentration estimated to be effective in reducing shell growth by 50%) for eastern oysters was 6 µg/ℓ (5-8 µg/ℓ); and the 96-hour LC50 for grass shrimp (Palaemonetes pugio) was 5 µg/ℓ (4-6 µg/ℓ). The 96-hour LC50's for saltwater fishes were 24 µg/ℓ (20-29 µg/ℓ) for sheepshead minnows and 6 µg/ℓ (5-7 µg/ℓ) for pinfish (Lagodon rhomboides). (All the median effect concentrations in both studies were based on measured concentrations of chlordane.)

In chronic tests with freshwater fishes, Cardwell et al. (op. cit.) reported "...the lowest concentrations of technical chlordane found to cause major chronic effects were 0.32 µg/ℓ for brook trout, 1.22 µg/ℓ for bluegill...." Neither a maximum acceptable toxicant concentration (MATC) nor application factor limits (Mount and Stephan, 1967) were reported for either fish species, however, because of experimental difficulties.

Trifluralin is a herbicide used extensively in agriculture. Sanders (1970) reported 48-hour LC50's for freshwater invertebrates in static tests as follows: waterflea, 560 µg/ℓ; seed shrimp (Cypridopis vidua), 250 µg/ℓ; scud (Gammarus fasciatus), 1,800 µg/ℓ; sowbug (Asellus brevicaudus), 2,000 µg/ℓ; grass shrimp (Palaemonetes kadiakensis), 1,200 µg/ℓ; and crayfish (Orconectes sp.), 50,000 µg/ℓ. Similar acute toxicities have been reported for other freshwater invertebrates (Sanders and Cope, 1966; 1968). Macek et al. (1969) reported that the acute toxicity of trifluralin increased with increased temperature. The 96-hour LC50 (and 95% confidence limits) for bluegills was 190 µg/ℓ (160-230 µg/ℓ) at 12.7 degrees Celsius (°C) and 47 µg/ℓ (40-55 µg/ℓ) at 23.8°C; the 96-hour LC50 for rainbow trout (Salmo gairdneri) was 210 µg/ℓ (182-240 µg/ℓ) at 1.6°C and 42 µg/ℓ (38-46 µg/ℓ) at 12.7°C, based on static tests.

Recently, Macek et al. (1976) reported the results of acute and chronic toxicity tests with trifluralin and waterfleas and fathead minnows. The 48-hour LC50 (and 95% confidence limits) for waterfleas was 193 µg/ℓ (155-327 µg/ℓ). The MATC was >2.4<7.4 µg/ℓ and the application factor (AP) limits were 0.012-0.037. For fathead minnows, the incipient LC50 (estimated after 12 days of exposure in a flow-through system) was 115 µg/ℓ with 95% confidence limits of 45-211 µg/ℓ. The MATC was >1.9<5.1 µg/ℓ and the AF limits (calculated by using the incipient LC50) were 0.017-0.044.

Pentachlorophenol is an organochlorine which is used extensively in this country and abroad in agricultural and industrial applications, particularly in wood processing (Bevenue and Beckman, 1967). Acute and sublethal effects of pentachlorophenol or its sodium salt on fresh- and saltwater invertebrates and

fishes have been reported (Crandall and Goodnight, 1962; Bandt and Nehring, 1962; Tomiyama and Kawbe, 1962; Tomiyama et al., 1962; Alderdice, 1963; Alabaster, 1969; Norup, 1972; Webb and Brett, 1973; and Kaila and Saarikoski, 1977). Except for the relatively resistant guppy (Poecilia reticulata) studies by Crandall and Goodnight (op. cit.) and the crayfish (Astacus fluviatilis) studied by Kaila and Saarikoski (op. cit.), acute median effect concentrations were from 170-800 µg/ℓ. Tagatz et al. (1977) found that pentachlorophenol affected the composition of communities of estuarine organisms at lower concentrations. Significantly fewer individuals and species were found in mean measured pentachlorophenol concentrations ≥76 µg/ℓ. Webb and Brett (op. cit.) reported sublethal effects in sockeye salmon (Oncorhynchus nerka) exposed to sodium pentachlorophenate. Growth rate and conversion efficiency were similarly affected, the median effect concentrations being 1.74 and 1.80 µg/ℓ, respectively. We know of no chronic fish studies with pentachlorophenol.

Our chronic tests were conducted during the following periods: chlordane, 6 September 1975-12 March 1976; trifluralin, 16 February-23 July 1976; and pentachlorophenol, 12 December 1975-8 June 1976.

SECTION 2

CONCLUSIONS

Sheepshead minnows (Cyprinodon variegatus) are suitable test animals for full life-cycle toxicity tests. Effects of chemicals on the reproductive portion of the life cycle and the critical life stages (embryo and juvenile) of the successive generation are readily determined.

Tests with this species of saltwater fish are practical means of determining maximum acceptable toxicant concentrations and application factors because of (a) the amenability of sheepshead minnows to laboratory culture and (b) the relatively short period of time required to reach sexual maturity and complete the full life cycle.

Results of chronic toxicity tests showed that sheepshead minnows were adversely affected by exposure to (a) 0.8 µg/ℓ chlordane; (b) 4.8 µg/ℓ trifluralin; or (c) 88 µg/ℓ pentachlorophenol. Therefore, exposure to concentrations equal to or greater than these would adversely affect the production of this saltwater fish species.

Measured concentrations of technical chlordane and trifluralin were consistently less than nominal, even though low concentrations of the solvents, acetone or triethylene glycol, were employed to aid dissolution and the toxicant solutions were continuously replenished. This indicates that toxicity tests with these insecticides would not be valid unless based upon measured concentrations of the chemicals. Measured concentrations of pentachlorophenol were near nominal.

The application factor limits derived for sheepshead minnows exposed to trifluralin were similar to those derived for a freshwater fish. Application factors derived for sheepshead minnows exposed to other pesticides have also been similar to those derived for freshwater fishes. This suggests that application factors, at least for pesticides, can be applied similarly to freshwater or saltwater fishes.

SECTION 3

RECOMMENDATIONS

Spawning groups comprising five sheepshead minnows in the ratio of 2 male fish:3 female fish are satisfactory to evaluate effects of a chemical on fecundity during a chronic exposure.

A 10-day spawning period for a spawning group is sufficient to monitor spawning success; at least two spawning periods should be monitored for all treatments if possible.

Survival of juvenile fish hatched from embryos from two different spawning periods should be monitored to assess potential cumulative effects of the toxicant.

Studies should be conducted with sheepshead minnows and other toxicants in full life-cycle tests because tests with this saltwater fish appear to provide accurate estimates of maximum acceptable toxicant concentrations in a shorter time and with less effort than do tests with most freshwater fishes.

SECTION 4

MATERIALS AND METHODS

TEST MATERIALS

The chlordane used in this study was a brown liquid, technical grade, 100% active ingredient and was obtained from Velsicol Chemical Corporation, 341 East Ohio Street, Chicago, Illinois 60611.

The trifluralin used was an orange powder, technical grade, 99% active ingredient (lot number 5GB70 X-14788) and was obtained from Eli Lilly and Company, Greenfield, Indiana.

The pentachlorophenol was purchased from J.T. Baker Chemical Company, Phillipsburg, New Jersey 08865, and was a fine, white powder, BAKER GRADE (lot number 416801).

Concentrations of each chemical are reported here as µg of the technical material described above per ℓ of seawater.

Stock solutions of each chemical were prepared on a weight: volume basis by adding appropriate amounts of chlordane to reagent grade acetone or trifluralin or pentachlorophenol to triethylene glycol. These 1-ℓ stock solutions were placed in amber glass bottles and stored in the laboratory. New stock solutions were prepared as required.

TEST WATER

All water used for holding, acclimation, and testing was natural seawater which was pumped from Big Lagoon into the laboratory. The pump intake was 85 meters (m) offshore at a depth of approximately 3 m. Water was pumped by a #316 stainless steel pump through hard polyvinylchloride (PVC) pipes, through a fiberglass, sand-filled filter, and through a 5-micrometer (µm) polypropylene bag filter into an elevated fiberglass reservoir. Water was continuously and vigorously aerated in the reservoir and flowed by gravity through PVC pipes to the diluters.

No attempt was made to alter the salinity of the water, but temperature was maintained at 30±1°C by heating the incoming seawater in the elevated reservoir with Teflon® tube-bundle heat exchangers and in small fiberglass-coated plywood boxes above the

diluters with electric quartz heaters. Test aquaria were also placed in constant-temperature water baths.

TEST ANIMALS

All sheepshead minnows used in these studies were collected from Big Lagoon, near Bionomics Marine Research Laboratory. They were held in sand-banked ponds on the laboratory grounds and in fiberglass or glass tanks in the laboratory. All fish were acclimated to test conditions for 14 days before testing according to the requirements of The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Mortality was <3% during acclimation. During holding and acclimation, fish were fed frozen adult or live nauplii of Artemia salina (San Francisco Bay Brand) or flaked commercial fish food (BiOrell® and Tetramin®) which contained no detectable concentrations (<0.1 µg/g) of chlorinated hydrocarbon pesticides or polychlorinated biphenyls as determined by our electron-capture gas chromatograph analyses.

Size of sheepshead minnows for the acute tests was as follows: chlordane--3.8-5.8 centimeters (cm) standard length (SL); trifluralin--1.0-1.5 cm SL; and pentachlorophenol--1.0-2.0 cm SL.

TEST METHODS

Acute Tests

All procedures followed methods of The Committee on Methods for Toxicity Tests with Aquatic Organisms (op. cit.) except as stated. The 96-hour tests were conducted in an intermittent-flow system by using a proportional diluter (Mount and Brungs, 1967) constructed to deliver 1 ℓ/cycle at a dilution ratio of 75%. The average number of cycles was 5/hour, providing approximately 99% replacement of water in the aquaria within 24 hours (Sprague, 1969). A mechanical injector (manufactured by George Frazer, Duluth, MN) equipped with a 50-milliliter (mℓ) glass syringe and stainless steel needle injected the appropriate volume of each chemical stock solution through polyethylene tubing to the mixing cell. Test containers were 30 x 30 x 61-cm glass aquaria. Each contained 20 fish and approximately 82 ℓ of water.

By continuing the acute exposure, a 21-day test was conducted with trifluralin in order to determine an incipient or threshold LC50 (Sprague, op. cit.)

Chronic Tests

Chronic tests were conducted as described above except that the proportional diluter was constructed for 50% dilution, test containers were duplicated for each treatment (separate aquaria were placed in an upper and lower water bath), and the diluter was modified (Schimmel et al., 1975) to include a solvent control

wherein the same volume of solvent/carrier (acetone or triethylene glycol) was added to chlordane-, trifluralin-, or pentachlorophenol-free seawater as was added to the highest chemical concentration. One injector, equipped with a 30-mℓ glass syringe and a stainless steel needle, metered the respective stock solutions through polyethylene tubing into the mixing chamber. A second injector, equipped with a 50-mℓ glass syringe with stainless steel needle, metered acetone or triethylene glycol to each solvent control. Maximum solvent concentration was 29 microliters (μℓ) per ℓ (parts per million, ppm).

To begin each test, eggs were stripped from adult fish whose egg production was enhanced by two injections of human chorionic gonadotrophin hormone (Schimmel et al, 1974). Testes were excised from males, macerated in seawater, and the resultant suspension was mixed with the eggs. Within 1 hour after visual confirmation of fertilization, a group of 50 embryos (two groups per duplicate treatment) was placed in an embryo incubator cup (a 100-mℓ glass jar with the bottom cut off and 480-μm square mesh nylon screen attached with silicone sealant). The incubator cups were suspended from a rocker-arm apparatus (Mount, 1968) which gently oscillated them in test aquaria. Embryos were removed from each cup by pipette daily, counted, and the cups were washed with bursts of fresh water to clean the screens. This procedure was repeated until all living embryos had hatched. Survival of embryos, length of time required for the embryos to hatch, and number of live fry were recorded. Then, 40 fry were impartially selected from each duplicate treatment and were placed in glass chambers (14-cm wide x 20.5-cm high x 26-cm long with 381-μm square mesh #316 stainless steel screen over one end). Fish were fed live brine shrimp nauplii or flaked commercial fish food ad libitum. Salinity and dissolved oxygen were measured daily. Light was provided by two 3.7-m fluorescent bulbs suspended 46 cm above the test containers, providing approximately 1,100 lux incident to the water surface. The photoperiod was 16 hours light and 8 hours dark. Survival was monitored daily by visually inspecting each test container, and any physical or behavioral changes were recorded. Growth was monitored biweekly according to the photographic method of McKim and Benoit (1971), and average weight was determined monthly by weighing each group in water.

When the fish were approximately 28 days old, the population was reduced to 20 fish per aquarium and released from the chambers into the aquaria. This number was chosen to ensure that sufficient fish would grow and mature for at least two spawning groups (each of which was comprised of five fish in the ratio of 2 males to 3 females) in each treatment.

The effects of each chemical on spawning were determined after fish began to exhibit signs of sexual maturity. Monitoring of spawning activity was begun on day 93 of the chlordane test, on day 113 of the trifluralin test, and on day 111 of the penta-

chlorophenol test. Spawning chambers were constructed by lacing pieces of 6.5-millimeter (mm) square mesh #316 stainless steel screen together with #316 stainless steel wire. The chambers were 30.6-cm wide x 30.5-cm long x 25.5-cm high, and were supported by 5-cm high extensions of the screen ends. Beneath each spawning chamber, a 29.5-cm square x 4.7-cm high collection tray was placed to retain the demersal eggs that sank through the bottom of the spawning chamber. The tray was constructed of plate glass and silicone sealant, with a 4-cm wide strip of 480-µm square mesh nylon screen along one side of the bottom to facilitate consolidation of eggs. Spawning groups, which consisted of two males and three females, were placed in the spawning chambers for a 10-day period. All possible 2:3 ratios in each aquarium were spawned once and extra, unspawned fish from each duplicate aquarium of a treatment were combined whenever possible to form additional spawning groups. Each day, one end of each spawning chamber was lifted slightly and the collection tray was removed from the aquarium. The eggs spawned and fertilized during the previous 24 hours were washed with seawater, transferred by large-bore glass pipette into glass Petri dishes, counted, and separated into groups of 50 embryos. Each 50-embryo group was then placed in an incubator cup as described above and placed in the same aquarium as the spawning group which produced it. The procedure described for initiating the tests was followed to monitor hatching and survival of second-generation juveniles. At least two groups of juveniles per duplicate from each treatment were monitored, except in the higher concentrations where toxicant-induced mortality made it impossible to obtain spawning groups and subsequent embryos and juveniles.

CHEMICAL ANALYSES

Seawater

For the chlordane and pentachlorophenol acute tests, water was collected from each aquarium at the beginning and end of the 96-hour exposure and for the acute trifluralin test, on days 1 and 21. Water was collected from alternate duplicate aquaria weekly during the chronic tests. Water samples were prepared and analyzed under the conditions described below.

Chlordane--

Unfiltered 800-mℓ seawater samples were extracted twice with 50-mℓ portions of Nanograde® dichloromethane. The combined extracts were dried with anhydrous sodium sulfate (previously heated at 500°C for 24 hours) and concentrated to approximately 1 mℓ in a Kuderna-Danish evaporative concentrator. The concentrated extracts were quantitatively transferred to a 15-mℓ centrifuge tube and evaporated to <0.5 mℓ by a gentle stream of clean, dry air. The samples were then diluted to appropriate volumes with Nanograde hexane, and an aliquot was withdrawn for analysis by gas liquid chromatography under the following conditions:

Instrument--Tracor Model MT-550 equipped with a Ni63 electron capture detector

Column--2-m x 2-millimeters (mm) (ID) Pyrex®, packed with 3% OV-101 on 100/120 mesh Supelcoport

Recorder--Corning Model 841 strip chart; 0-1 mV full scale

Temperatures (°C)--Column: 200 Outlet: 245
Inlet: 230 Detector: 305

Gas flows (cubic centimeters [cc] of nitrogen/min [min]--Carrier: 40
Make up: 48

Chart speed--0.5 cm/min

Attenuation--8 x 100

Retention times--4.1, 4.6, 5.5, 6.6, 8.2 (main peak), 9.1, 9.5 (shoulder), and 14.2 min

Response--Half-scale chart deflection at 8.2 min. for 3.0 nanograms (ng) of chlordane

Calibration curves were obtained by plotting the sum of the peak heights (in mm) at 4.1, 4.6, 5.5, 6.6, 8.2, 9.1, 9.5, and 14.2 min against the weight (ng) of chlordane analyzed. According to the National Research Council of Canada (1975), quantitation of chlordane GLC profiles by peak height summation or by peak area summation produces comparable accuracy. A tangent skim correction was applied to all samples in which peaks eluted on the tail of the solvent.

Trifluralin--

Unfiltered 500-mℓ seawater samples were extracted twice with 50-mℓ of Nanograde dichloromethane. The extracts were dried with anhydrous sodium sulfate (previously heated at 500°C for 24 hours), filtered, combined into a Kuderna-Danish evaporative concentrator, and concentrated to approximately 3 mℓ. The concentrated extracts were quantitatively transferred to a 10-mℓ graduated mixing tube and evaporated to <0.5 mℓ by a gentle stream of clean, dry air. The samples were then diluted to 10.0 mℓ with Nanograde hexane. Approximately 1 mℓ was removed from each sample and sealed in a septum vial for gas chromatographic analysis under the following conditions:

Instrument--Hewlett-Packard 5840A gas chromatograph equipped with a Ni63 electron capture detector and a Model 7671A automatic sampler

Column--2 m x 2 mm (ID) Pyrex packed with 5% OV-17 on 100/120 mesh Chromosorb G.H.P.

Temperatures (°C)--Column: 240
Inlet: 260
Detector: 330

Gas flow--Carrier: 30 cc nitrogen/min
Make-up: 20 cc 95% argon:5% methane/min

Set points--Slope sensitivity: 0.75-0.20
Area rejection: 50,000 counts
Cycle time: 8.0 min
Calibration method: external standard
Percent retention window: 5.0

Retention time--3.71-3.76 min

Minimum detectable concentration--0.36 µg/ℓ when using a 500-mℓ water sample

Daily instrument calibration was performed by using 0.5, 1.0, and 1.5 µg/mℓ trifluralin-in-hexane standards, respectively, dependent upon the expected concentration range of samples. Standards were checked every eighth sample during an automatic sampling sequence.

Extraction and analysis efficiency were determined as follows: samples were produced by adding 1.0 mℓ of a 5.0 and 0.5 µg/mℓ trifluralin-in-acetone solution to 500-mℓ volumes of synthetic seawater to obtain concentrations of 10.0 µg/ℓ and 1.0 µg/ℓ trifluralin, respectively. Unfortified samples of synthetic seawater (500 mℓ) were used as blanks. All samples were extracted and analyzed according to the above procedure with the following results.

Trifluralin added (µg/ℓ)	Trifluralin recovered (µg/ℓ)	Percentage recovery
10.0	11	110
10.0	12	120
10.0	14	140
1.0	1.0	100
1.0	1.1	110
1.0	1.2	120
0.0	<0.36	---
0.0	<0.36	---
0.0	<0.36	---

Pentachlorophenol--

The methodology for the analysis of pentachlorophenol in seawater was modified from an EPA (1974) procedure for the

analysis of pentachlorophenol in urine and water.

Unfiltered 100-mℓ seawater samples were measured volumetrically, transferred to a 250-mℓ separatory funnel, acidified with 4 mℓ of reagent grade concentrated sulfuric acid, and extracted with 30 mℓ of Nanograde hexane by vigorously shaking the contents of the funnel for approximately two minutes. The seawater was discarded and the hexane extract was transferred into a small amber glass bottle equipped with a Teflon-lined screw cap.

An ethereal solution of diazomethane (N-methyl-N-nitroso-p-toluenesulfonamide) was prepared according to Aldrich Chemical Company's Bulletin Z10, 025-0. This solution was added to a potassium hydroxide-water-ethanol mixture; the ethyl ether was distilled and collected at -60°C until the distillate was colorless. The ethereal distillate contained approximatley 3 g of diazomethane in approximately 200 mℓ of ethyl ether. All batches of diazomethane were stored at -60°C in a high-draft air exhaust hood throughout the analyses.

A 0.5-mℓ aliquot of the 30-mℓ hexane extract was added by pipette to a 15-mℓ centrifuge tube equipped with a Teflon-lined screw cap. Then, 0.1 mℓ of the diazomethane solution was added. The tube was capped immediately, the contents were mixed for one minute by using a Vortex® Genie mixer, and the tube and contents stored at room temperature for twenty minutes. After methylation, 1 mℓ of 20% distilled water-in-Nanograde methanol was added and the tube was shaken by the Vortex mixer for two minutes to destroy the excess diazomethane. The phase separation between hexane (top layer) and the water-methanol (bottom layer) was immediate and no emulsification occurred. An aliquot of the hexane layer was removed and analyzed for pentachlorophenol by gas-liquid chromatography (GLC).

The methylated pentachlorophenol-hexane extracts were diluted with Nanograde hexane prior to GLC analysis as follows:

Nominal concentration of pentachlorophenol in seawater (µg/ℓ)	Concentration of pentachlorophenol in hexane extract (µg/mℓ)	Extract volume (mℓ)	Hexane added (mℓ)	Dilution factor	Expected pentachlorophenol concentration (µg/mℓ)
400	1.33	0.5	39.5	80	0.0167
200	0.667	0.5	19.5	40	0.0167
100	0.333	0.5	9.5	20	0.0167
50	0.167	0.5	4.5	10	0.0167
25	0.0833	0.5	2.0	5	0.0167
Control	---	0.5	0	---	---

All sample and standard injections were identical (2 µℓ)

throughout the analytical period. In this manner, the effect of varying volumes of solvent upon the chromatogram of pentachlorophenol was eliminated, and the analyses of all seawater (except control samples) were performed with identical GLC instrumental conditions.

The GLC operating conditions were as follows:

Instrument--Perkin-Elmer Model 3920 equipped with a 1-m x 2-mm (ID) Pyrex column packed with 5% (by weight) OV-101 on 100/120 mesh Chromasorb W, HP

Detector--Electron capture with 15 mC Ni63

Temperatures (°C)--Inlet: 220 Outlet: 240
Column: 150 Detector: 300

Gas flow--Carrier: 28 cc nitrogen/min
Make-up: 55 cc nitrogen/min

Recorder--0-1 mV sensitivity, 0.677 cm/min chart speed

Response--10 picograms of pentachlorophenol gave 86% and 15% of full-scale recorder response with an electrometer attenuation of 16X and 64X, respectively. Pentachlorophenol eluted in 1.55 minutes.

Nanograde hexane (up to 6 µℓ) gave no pentachlorophenol response. In addition, a series of reagent blanks prepared by methylating 0.5 mℓ of hexane produced a consistent pentachlorophenol response equivalent to 0.013 µg pentachlorophenol/ℓ of seawater. This background of pentachlorophenol contamination was subtracted only from the control seawater samples.

According to EPA (1974), the hexane extraction of acidified urine removes at least 90% of the dissolved pentachlorophenol; the methylation reaction is complete over large concentration ranges of both pentachlorophenol and diazomethane; and finally, the methanol/water rinse does not extract methylated pentachlorophenol from the hexane layer. In view of the expected high recovery of pentachlorophenol by the above method, we prepared 100-mℓ portions of synthetic seawater containing known concentrations of pentachlorophenol. The seawater was extracted, methylated, and diluted with hexane to provide the pentachlorophenol standards which were used to calibrate the response of the chromatograph to unknown pentachlorophenol concentrations. A graph of the GLC peak height versus the concentration of pentachlorophenol in the standard synthetic seawater was constructed and all sample concentrations were obtained by interpolating the sample peak heights to the corresponding pentachlorophenol concentration.

Extraction efficiency and mean recovery for the analytical

methods were 86.5±8.0% for chlordane and 116.3±11.2% for trifluralin. Data from chlordane analyses have been corrected for recovery; trifluralin data were based on 100% recovery.

Fish Tissue

Fish were collected for residue analyses as follows:

a. adults alive at the end of the respective exposure;
b. juveniles alive at the end of the 28-day growth period; and
c. eggs and embryos randomly collected during the spawning periods.

Chlordane--

Tissue samples were prepared for gas chromatographic analysis by blending the sample in methylene chloride for 30 seconds using a Polytron® homogenizer. The extract was filtered through anhydrous sodium sulfate into a Kuderna-Danish evaporative concentrator and concentrated to approximately 3 mℓ. The extract was evaporated to dryness using a gentle stream of clean, dry air and immediately diluted with a 1.0 mℓ of Nanograde hexane for florisil column adsorption chromatography.

The concentrate was quantitatively transferred with hexane to a 10 x 300-mm Pyrex chromatographic column containing a 10.2-cm layer of activated (130°C) florisil 60/100 mesh with a 2.5-cm layer of anhydrous sodium sulfate above it. The column was prewashed with petroleum ether and the solvent was adjusted to within 1 cm above the upper sodium sulfate layer before sample addition. Chlordane was eluted from the column with 100 mℓ of 6% peroxide-free ethyl ether (EPA, 1974) in petroleum ether with a flow rate of 5 mℓ per minute.

The eluate was concentrated in a Kuderna-Danish evaporative concentrator to approximately 3 mℓ. The extract was transferred to a 15-mℓ centrifuge tube and evaporated to dryness with air. The residue was then dissolved in hexane for analysis by gas liquid chromatography with the following instrumental conditions:

Instrument--Tracor Model MT-550 equipped with a Ni63 electron capture detector

Column--2-m x 2-mm (ID) Pyrex packed with 3% OV-101 on 100/120 mesh Supelcoport

Recorder--Corning Model 841 strip chart; 0-1 mV full-scale

Temperatures (°C)--Column: 200 Outlet: 245
Inlet: 230 Detector: 305

Gas flows--Carrier: 40 cc nitrogen/min
Make-up: 48 cc nitrogen/min

Chart speed--0.5 cm/min

Attenuation--8 x 100

Retention times--4.1, 4.6, 5.5, 6.6, 8.2 (main peak), 9.1, 9.5 (shoulder), 14.2 min

Response--Half scale chart deflection at 8.2 min for 3.0 ng chlordane

Calibration curves were obtained by plotting the sum of the peak heights at 4.1, 4.6, 5.5, 6.6, 8.2, 9.1, 9.5, and 14.2 minutes against the weight (ng) of chlordane injected. According to The National Research Council of Canada (1975), quantitation of chlordane GLC profiles by peak height summation or by peak area summation produces comparable accuracy. A tangent skim correction was applied to all samples in which peaks eluted on the tail of the solvent.

To determine extraction and analysis efficiency, three samples were produced by adding 1.0 mℓ of a 1.0 ppm solution of chlordane in acetone to each tissue sample. The samples were analyzed by the above method with the following results.

Tissue weight (g)	Chlordane added (µg/g)	Chlordane recovered (µg/g)	Percentage recovery
0.6343	1.6	1.5	94
0.7905	1.3	1.2	92
1.1228	0.89	0.81	91

The minimum detectable concentration of chlordane in tissue was 0.1 µg/g when using a 1-g sample.

Trifluralin--

Weighed groups of whole fish (total weight <10 g) were homogenized with a 25-mℓ volume of 1:1 petroleum ether-diethyl ether by using a PT-10 Willems Polytron homogenizer. The homogenate was centrifuged and the mixed ether phase was decanted and saved. An additional 25-mℓ volume of 1:1 petroleum ether-diethyl ether was added to the homogenate and the extraction repeated. The two ether extracts were combined, transferred to a Kuderna-Danish evaporative concentrator, and concentrated to approximately 3 mℓ. The extract was transferred with hexane to a separatory funnel, the volume was diluted to 15 mℓ of hexane, and the mixture was extracted 4 times with 30-mℓ volumes of acetonitrile. The acetonitrile phases were combined and transferred to a separatory funnel containing a 650-mℓ volume of distilled water and a 40-mℓ volume of saturated sodium chloride solution. The mixture was extracted twice with 100-mℓ volumes of hexane. The hexane extracts were combined in a separatory funnel, and the hexane was washed

twice with 100-mℓ volumes of distilled water. The water was discarded and the hexane was transferred to a column containing an 8- to 10-cm layer of anhydrous sodium sulfate. The separatory funnel and column were rinsed three times with 10-mℓ volumes of hexane. The hexane was combined and collected in a Kuderna-Danish evaporative concentrator, and concentrated to approximately 3 mℓ. The extract was quantitatively diluted to an appropriate volume for gas chromatographic analysis under the following instrumental conditions:

Instrument--Hewlett-Packard 5840A gas chromatograph equipped with a 15 mC Ni63 electron capture detector and a Model 7671A automatic sampler

Column--3-m x 2-mm (ID) Pyrex, packed with 5% OV-17 on 100/120 mesh Chromosorb G.H.P.

Temperatures (°C)--Column: 240
Inlet: 260
Detector: 330

Gas flow--Carrier: 30 cc nitrogen/min
Make-up: 20 cc 95% argon:5% methane/min

Set points--Slope sensitivity: 200
Area rejection: 10,000 counts
Cycle time: 6.0 min
Calibration method: External standard
Percent retention window: 5.0

Retention time--3.85 min.

Daily instrument calibration was performed with 0.1 and 1.0 µg/mℓ trifluralin in hexane standards. Standards were checked after every eighth sample during an automatic sampling sequence.

To determine extraction and analysis efficiency, samples were produced by adding 5 mℓ of a 0.5 µg/mℓ or 5 mℓ of a 5.0 µg/mℓ trifluralin-in-acetone standard to 5.0 g of uncontaminated fish samples. The fortified fish samples were extracted and analyzed according to the above procedure with the following results.

Trifluralin added (µg)	Trifluralin recovered (µg)	Percentage recovery
0	<0.4	---
0	<0.4	---
0	<0.4	---
2.5	1.62	65
2.5	1.76	70
2.5	1.84	73
25.0	18.1	72
25.0	15.7	63
25.0	16.3	65

The minimum detectable concentration of trifluralin in tissue was 0.4 µg.

Pentachlorophenol--

Wet fish tissue was weighed, transferred with a 0.4 normal sodium hydroxide solution to an appropriate vessel for grinding, and homogenized with a Polytron PT-10/20. The homogenizer blades were rinsed with a portion of distilled water, and the rinse was added to the sample. Samples weighing <2 g were extracted with 5 mℓ of 0.4 normal sodium hydroxide, and the blades were rinsed with 5 mℓ of distilled water. Samples weighing 2-7 g were extracted with 12.5 mℓ of 0.4 normal sodium hydroxide and the blades were rinsed with 12.5 mℓ of distilled water.

Two mℓ of the basic aqueous extract containing pentachlorophenol were extracted with 5 mℓ of Nanograde hexane to remove the majority of fats and oils, and the hexane was discarded. The aqueous phase was acidified to pH 2 by adding concentrated hydrochloric acid and extracted with an additional 2 mℓ of Nanograde hexane by a Vortex Genie tube shaker. The stable emulsion formed between hexane and the aqueous acid extract was separated by centrifuging at 3,600 rpm for 20 minutes in a 20-cm diameter centrifuge head.

After phase separation, 0.5 mℓ of the hexane was transferred to a clean, Teflon-capped tube and methylated with diazomethane, according to EPA (1974). Solutions containing known weights of pentachlorophenol (Chemical Service Company, Catalog Number 7) were methylated and used to standardize the gas chromatographic response to known pentachlorophenol weights. Aliquots of the methylated pentachlorophenol in hexane were analyzed by gas chromatography using the identical instrumental conditions previously described for the analysis of pentachlorophenol in water.

The percentage recovery of pentachlorophenol from fish tissue was determined by adding 0.5 µg of pentachlorophenol to each of three 2-g tissue samples. The pentachlorophenol was extracted, methylated, and analyzed as described above with the following results.

Pentachlorophenol added (µg/g)	Pentachlorophenol recovered (µg/g)	Percentage recovery
0.25	0.19	76
0.25	0.19	76
0.25	0.22	88

Due to the prevalence of pentachlorophenol contamination in an analytical chemistry laboratory environment, three reagent blanks were analyzed and found to contain 2.1 ng of pentachlorophenol per blank. The pentachlorophenol due to reagents and solvents

was subtracted from all tissue samples reported.

Extraction efficiency and mean recovery for the analytical methods were 92.3±1.0% for chlordane; 68.2±4.5% for trifluralin; and 80.0±6.9% for pentachlorophenol. Data reported have been corrected for recovery.

STATISTICAL ANALYSES

In the acute tests, the LC50's and 95% confidence limits were calculated by linear regression analysis after probit transformation (Finney, 1971).

In the chronic tests, differences between treatments were determined by analysis of variance (Sokol and Rohlf, 1973). Differences were considered significant at the 95% ($P<0.05$) confidence level. Post-hoc tests were conducted on treatment means by using Dunnett's procedure (Steel and Torrie, 1960).

The control was compared to all other treatments (including the solvent control) in all tests. In two instances there was a significant difference between the control and the solvent control--see the chlordane and trifluralin results and discussion sections for details.

SECTION 5

RESULTS AND DISCUSSION

CHEMICAL ANALYSES

Mean measured concentrations of chlordane in seawater were from 10-15% of nominal during the acute test and from 19-60% of nominal during the chronic test (TABLE 1), reflecting the low solubility of this chlorinated hydrocarbon pesticide in water.

TABLE 1. NOMINAL AND MEASURED CONCENTRATIONS OF CHLORDANE DURING ACUTE AND CHRONIC EXPOSURES OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) IN FLOWING, NATURAL SEAWATER.

Test	Nominal	Concentration (µg/ℓ) Measured 0 hour	96 hour	Avg.	% of nom.	
ACUTE	Control	ND[a]	ND	---	---	
	18	1.3	2.4	1.8	10	
	23	2.6	2.7	2.6	11	
	31	3.8	4.3	4.0	13	
	42	6.6	5.9	6.2	15	
	56	-[a]	6.7	6.7	12	
	75	12.0	3.4	7.7	10	
	100	12.0	18.0	15.0	15	
		Mean	S.D.	Range	% of nom.	# samples
CHRONIC	Control	ND[a]	---	---	---	18
	Sol. control	-[b]	-	-	-	-
	1.9	0.5	±0.4	ND-1.7	26	13
	3.8	0.8	±0.6	ND-2.3	21	14
	7.5	1.7	±1.1	0.4-4.4	23	17
	15.0	2.8	±1.8	0.9-8.0	19	16
	30.0	18.0	0.0	---	60	2

[a]Not detectable; <0.23 µg/ℓ.
[b]Not analyzed.

Mean measured concentrations of trifluralin in seawater were from 47-56% of nominal during the acute test and from 21-38% of nominal during the chronic test (TABLE 2).

TABLE 2. NOMINAL AND MEASURED CONCENTRATIONS OF TRIFLURALIN DURING ACUTE AND CHRONIC EXPOSURES OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) IN FLOWING, NATURAL SEAWATER.

Test	Nominal	Concentration (µg/ℓ) Measured			
		Day 1	Day 21	Avg.	% of nom.
ACUTE	Control	0.48	0.78	0.63	—
	107	60	48	54	50
	142	86	66	76	54
	190	124	86	105	55
	253	162	100	131	52
	337	216	162	189	56
	450	250	176	213	47
	600	368	268	318	53

		Mean	S.D.	Range	% of nom.	# samples
CHRONIC	Control	ND[a]	—	—	—	23
	Sol. control	—[b]	-	-	-	-
	6.3	1.3	±0.6	ND-3.1	21	21
	12.5	4.8	±2.3	1.2-10.2	38	23
	25.0	9.6	±6.1	4.1-32.0	38	23
	50.0	17.7	±6.5	6.6-31.0	35	23
	100.0	34.1	±15.2	13.0-65.0	34	16

[a]Not detectable; <0.36 µg/ℓ.
[b]Not analyzed.

Mean measured concentrations of pentachlorophenol were from 84-110% of nominal during the acute test and from 72-98% of nominal during the chronic test (TABLE 3).

TABLE 3. NOMINAL AND MEASURED CONCENTRATIONS OF PENTACHLOROPHENOL DURING ACUTE AND CHRONIC EXPOSURES OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) IN FLOWING, NATURAL SEAWATER.

Test	Concentration (µg/ℓ) Nominal	Measured 0 hour	Measured 96 hour	Measured Avg.	Measured % of nom.
ACUTE	Control	ND[a]	0.04	---	---
	142	-[b]	-	-	-
	190	-[b]	-	-	-
	253	290	240	265	105
	337	370	-[a]	370	110
	450	480	490	485	108
	600	460	545	502	84

		Mean	S.D.	Range	% of nom.	# samples
CHRONIC	Control	0.2	±0.3	ND-0.9	---	24
	Sol. control	-[b]	-	-	-	-
	25	18	±6	9-36	72	22
	50	47	±10	23-65	94	24
	100	88	±21	42-136	88	23
	200	195	±42	80-270	98	24
	400	389	±78	290-510	97	7

[a]Not detectable; <0.04 µg/ℓ.
[b]Not analyzed.

A special analysis of the pentachlorophenol was conducted in order to identify the presence of certain impurities. The analyses was by mass spectroscopy and revealed the following:

Impurity	Concentration (µg/g; parts per million)
Tetrachlorophenol	3,000
Hexachlorodibenzodioxin	<1
Octachlorodibenzodioxin	1

Not enough of the pentachlorophenol sample remained for analyses for other impurities (personal communication, Dean R. Branson[a]).

[a]Dean R. Branson, Ph.D., personal communication, December 1977, Dow Chemical Company, Midland, MI 48640.

ACUTE TOXICITY

The acute toxicity of trifluralin and pentachlorophenol to sheepshead minnows was similar; chlordane was one order of magnitude more toxic (TABLES 4-5).

TABLE 4. TOXICITY OF THREE CHEMICALS TO SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 96 HOURS IN FLOWING, NATURAL SEAWATER.

Chemical	Measured concentration (µg/ℓ)	Mortality (%)
Chlordane[a]	Control	0
	1.8	0
	2.6	0
	4.0	0
	6.2	0
	6.7	0
	7.7	20
	15.0	70
Trifluralin[b]	Control	0
	54	0
	76	5
	105	15
	131	15
	189	30
	213	50
	318	95
Pentachlorophenol[c]	Control	0
	142[d]	0
	190[d]	0
	265	10
	370	10
	485	40
	502	90

[a]Salinity was 26 °/oo and temperature was 28°C.
[b]Salinity was 21 °/oo and temperature was 29°C.
[c]Salinity was 24 °/oo and temperature was 29°C.
[d]Nominal concentration.

TABLE 5. CALCULATED 96-HOUR LC50's FOR THREE CHEMICALS AND SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS). CALCULATIONS WERE BASED ON MEASURED CONCENTRATIONS OF EACH CHEMICAL.

Chemical	96-hour LC50 (µg/ℓ)	95% confidence limits (µg/ℓ)
Chlordane	12.5	3.4-45.9
Trifluralin	190	128-282
Pentachlorophenol	442	308-635

The acute toxicity of chlordane to sheepshead minnows tested under dynamic conditions was similar to that reported for other estuarine fishes tested under dynamic conditions (Parrish et al., 1976) and slightly greater than the toxicity reported for freshwater fishes tested under dynamic conditions (Cardwell et al., 1977) or static conditions (Henderson et al., 1959). The 96-hour LC50's for two saltwater fishes were 24.5 and 6.4 µg/ℓ, and the range of LC50's for seven freshwater fishes was 36.9-190 µg/ℓ.

Sheepshead minnows were not as sensitive to trifluralin in acute tests as were two freshwater fishes. Macek et al. (1969) reported a 96-hour LC50 of 47 µg/ℓ for bluegill tested at 23.8°C and a 96-hour LC50 of 42 µg/ℓ for rainbow trout tested at 12.7°C.

The incipient or threshold LC50 of trifluralin to sheepshead minnows was estimated after 10 days of exposure and was 84 µg/ℓ, with 95% confidence limits of 48-145 µg/ℓ. Macek et al. (1976) reported the incipient LC50 of trifluralin to fathead minnows (estimated after 12 days of exposure) to be 115 µg/ℓ, with 95% confidence limits of 45-211 µg/ℓ.

The susceptibility of sheepshead minnows to pentachlorophenol cannot be related to the susceptibility of other fishes to this chemical because few references exist in the literature. Data exist concerning the effects of sodium pentachlorophenate on fishes, but a comparison of the toxicity of the two chemicals may not be valid because of the differing chemical characteristics, particularly the effect of pH on toxicity (Kaila and Saarikoski, 1977). However, Davis and Hoos (1975) reported 96-hour LC50's for sodium pentachlorophenate and rainbow trout, coho salmon (Oncorhynchus kisutch), and sockeye (O. nerka) ranging from 37-130 µg/ℓ. The static tests were conducted in freshwater, initial pH 5.7-9.0. Schimmel et al. (in press) reported the 96-hour LC50 of sodium pentachlorophenate for three saltwater fishes: longnose killifish (Fundulus similis) >306 µg/ℓ; pinfish, 53 µg/ℓ; and

striped mullet (Mugil cephalus), 112 µg/ℓ. The tests were continuous flow and the concentrations were measured.

CHRONIC TOXICITY

Chlordane

Exposure to chlordane significantly reduced survival of parental fish during the 189-day study. All fish exposed to 18 µg/ℓ died within 30 days, and mortality of fish exposed to 2.8 µg/ℓ was significantly greater than mortality of control fish at the end of the study (TABLE 6).

Growth of parental fish after 34, 94, and 189 days of exposure to chlordane was not significantly different from growth of control fish (TABLE A-1).

The total number of eggs spawned by parental fish in the solvent control and all chlordane concentrations was significantly different from the control. The number of eggs spawned by fish in the solvent control and fish exposed to 0.5 µg/ℓ of chlordane was significantly greater than the number of eggs spawned by control fish, but the number of eggs spawned by fish exposed to chlordane concentrations ≥0.8 µg/ℓ was significantly less than the number of eggs spawned by the control or solvent control fish (TABLE 7). Because female fish died or were killed by male fish in spawning chambers in the control and three concentrations, we calculated eggs per female spawning day. These values were obtained by dividing the number of eggs obtained from a spawning chamber during a 24-hour period by the number of live female fish in the spawning chamber during the same period. The same pattern of effect observed for total eggs spawned was evident: more eggs were spawned per day per female in the solvent control and 0.5 µg/ℓ of chlordane than were spawned in the control; fewer eggs were spawned in concentrations ≥0.8 µg/ℓ but the differences were not significant (TABLE 8). Therefore, fecundity was not considered to be significantly affected by exposure to chlordane concentrations ≤2.8 µg/ℓ.

Hatching success of juveniles from embryos produced by fish exposed to chlordane decreased as exposure duration of parental fish increased. For the first and second spawning periods, hatching success in only one concentration (2.8 µg/ℓ) was affected. For the last spawning period, however, hatching success in two lower concentrations (0.8 and 1.7 µg/ℓ) was significantly less than in the control (TABLE 9).

Similarly, juvenile mortality during 28 days posthatch increased with increased duration of exposure of parental fish to chlordane. Mortality of exposed juveniles from the first spawning period was not significantly greater than mortality of control juveniles in any concentration, but mortality of juveniles from the second spawning period was significantly greater in two concen-

trations (1.7 and 2.8 µg/ℓ). Growth of surviving juveniles was not affected in any concentration, however (TABLE 10).

TABLE 6. CUMULATIVE MORTALITY (%) OF PARENTAL SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO CHLORDANE IN FLOWING, NATURAL SEAWATER. MORTALITY IS THE AVERAGE FROM DUPLICATE AQUARIA AND DOES NOT INCLUDE DEATHS WHICH OCCURRED IN THE SPAWNING CHAMBERS.

	Mean measured concentration (µg/ℓ)						
Day	Control	Solvent control	0.5	0.8	1.7	2.8	18.0
1-30	0	0	0	0	0	6	100
31-60	0	0	0	0	0	0	---
61-90	0	0	0	0	0	0	---
91-120	0	0	0	0	0	8	---
121-150	0	0	2	2	2	2	---
151-189	0	0	0	2	8	18	---
TOTAL	0	0	2	4	10	34[a]	100[a]

[a]Significantly greater than the control.

TABLE 7. NUMBER OF EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) DURING THREE 10-DAY SPAWNING PERIODS; FISH WERE EXPOSED TO CHLORDANE IN FLOWING, NATURAL SEAWATER FOR 189 DAYS. FIVE FISH (2 MALES AND 3 FEMALES) WERE PLACED IN A SPAWNING CHAMBER IN DUPLICATE AQUARIA, A AND B.

Day	Mean measured concentration (µg/ℓ)											
	Control		Solvent Control		0.5		0.8		1.7		2.8	
	A	B	A	B	A	B	A	B	A	B	A	B
93 to 102	264	182[a]	385	398	443	454	176	259	244	76[a]	67	524
139 to 148	412[a]	372	338	287	103[a]	387	327[a]	167[a]	462	88	247	135
177 to 186	127	269	404	691	392	655	167[a]	310[a]	208	121[a]	-[b]	28
SUB-TOTAL	803	823	1,127	1,376	938	1,496	670	736	914	285	314	687
TOTAL	1,626		2,503[c]		2,434[c]		1,406[d]		1,199[d]		1,001[d]	

[a]Death
[b]No spawning chamber; all surviving fish had been spawned.
[c]Significantly greater than the control.
[d]Significantly less than the control.

TABLE 8. NUMBER OF EGGS SPAWNED PER DAY PER FEMALE SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) DURING THREE 10-DAY SPAWNING PERIODS; FISH WERE EXPOSED TO CHLORDANE IN FLOWING, NATURAL SEAWATER FOR 189 DAYS. FIVE UNSPAWNED FISH (2 MALES AND 3 FEMALES) WERE PLACED IN A SPAWNING CHAMBER IN DUPLICATE AQUARIA, A AND B.

Day	Mean measured concentration (µg/ℓ)											
	Control		Solvent Control		0.5		0.8		1.7		2.8	
	A	B	A	B	A	B	A	B	A	B	A	B
93-102	9	10	13	13	15	15	6	9	8	3	3	17
139-148	16	12	11	10	5	14	11	6	15	3	8	4
177-186	4	9	14	23	13	22	6	11	7	4	-[a]	21
Mean of duplicate	10	10	13	15	11	17	8	9	10	3	6	7
Mean and S.D. of treatment	10±4		14±5		14±5		8±2		7±5		7±6	

[a]No spawning chamber; all surviving fish had been spawned.

TABLE 9. HATCHING SUCCESS OF JUVENILES FROM EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO CHLORDANE IN FLOWING, NATURAL SEAWATER. PERCENTAGE HATCH AND STANDARD DEVIATION REPRESENT POOLED DATA FROM DUPLICATE AQUARIA DURING THREE 10-DAY SPAWNING PERIODS.

Mean measured concentration (µg/ℓ)	Percentage hatch and S.D.				Number of embryos examined
	1st	2nd	3rd	Mean	
Control	99±1	99±1	99±1	99±1	638
Sol. control	99±1	98±3	99±1	99±2	1,089
0.5	98±2	97±1	97±3	98±2	1,151
0.8	96±3	98±3	74±21[a]	85±19	596
1.7	98±0	99±1	72±32[a]	84±26	565
2.8	90±2[a]	81±10[a]	83[b]	85±8	472

[a]Significantly less than the control.
[b]Insufficient data for statistical analysis; only one group of embryos.

TABLE 10. PERCENTAGE MORTALITY, AVERAGE STANDARD LENGTH, AND AVERAGE WEIGHT (DETERMINED IN WATER) OF 28-DAY OLD SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) JUVENILES HATCHED FROM EGGS SPAWNED BY FISH EXPOSED TO CHLORDANE FOR 88-91 DAYS (GROUP 1) AND 139-148 DAYS (GROUP 2).

Mean measured concentration (µg/ℓ)	GROUP 1				GROUP 2			
	Number of juv.	Mortality (%)	Length and S.D. (cm)	Weight (g)	Number of juv.	Mortality (%)	Length and S.D. (cm)	Weight (g)
Control	80	4	1.20±0.13	0.05	120	0	1.12±0.16	0.03
Sol. control	120	4	1.22±0.16	0.05	80	1	1.09±0.12	0.03
0.5	160	3	1.17±0.14	0.05	40	0	1.19±0.12	0.03
0.8	80	4	1.12±0.13	0.05	80	10	1.19±0.16	0.04
1.7	80	1	1.18±0.24	0.05	80	22[a]	1.09±0.19	0.03
2.8	80	12	1.24±0.14	0.05	80	29[a]	1.09±0.17	0.03

[a]Significantly greater than the control.

Trifluralin

Exposure to trifluralin significantly decreased survival of parental fish during the 166-day study. All fish exposed to 34.1 µg/ℓ died and 66% of the fish exposed to 17.7 µg/ℓ died, a significantly greater percentage than control mortality (TABLE 11).

Several trifluralin induced effects were observed. As early as day 14 posthatch, juvenile fish in concentrations ≥17.7 µg/ℓ were in poor condition. They exhibited loss of equilibrium and lay on the bottom of the aquaria with opercular movement only. Fish in two lower concentrations (4.8 and 9.6 µg/ℓ) had darkened coloration in the caudal peduncle area. On day 37, fish in 17.7 µg/ℓ were noticeably "fatter" than control fish. This edematous condition persisted throughout the exposure. On day 53, approximately one-fourth of the body of surviving fish in 17.7 µg/ℓ was darkened, the fish were extremely sluggish, and they exhibited loss of equilibrium, occasionally swimming upside down. Fish still alive in 34.1 µg/ℓ exhibited complete loss of equilibrium. On day 129 (12 days posthatch), F_1 juveniles in 17.7 µg/ℓ were sluggish and exhibited loss of equilibrium.

Exposure to trifluralin caused decreased growth of parental fish. Surviving fish in 34.1 µg/ℓ were less than half the length of control fish on day 37, for example, and fish in 9.6 and 17.7 µg/ℓ were significantly smaller than control fish at the end of the exposure (TABLE 12).

The total number of eggs spawned by parental fish in the solvent control and all trifluralin concentrations was significantly less than the number of eggs spawned by parental control fish (TABLE 13). Because female fish died or were killed by aggressive males during spawning in all treatments except 17.7 µg/ℓ, we calculated eggs per female spawning day. The same pattern of effect observed for total eggs spawned was evident: fewer eggs were spawned per day per female in all treatments than were spawned in the control. However, significantly fewer eggs were spawned only in trifluralin concentrations ≥4.8 µg/ℓ (TABLE 14). Therefore, fecundity (eggs per female per spawning day) was considered to be significantly affected by exposure to trifluralin concentrations ≥4.8 µg/ℓ.

Exposure to trifluralin decreased hatching success of juveniles from embryos produced by parental fish. Further, hatching success decreased as exposure duration of parental fish increased. For example, the mean hatch of juveniles in 9.6 µg/ℓ was 98% for the first spawning period. Hatch decreased to 54% for the second spawning period, however, and no juveniles hatched from embryos produced during the third spawning period (TABLE 15).

Trifluralin also adversely affected juvenile survival and growth. Mortality increased as concentration increased, with maximum mortality of 67% in 17.7 µg/ℓ compared to 14% in the control. Length of surviving juveniles in 9.6 µg/ℓ was significantly less than length of control juveniles (TABLE 16).

TABLE 11. CUMULATIVE MORTALITY (%) OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO TRIFLURALIN IN FLOWING, NATURAL SEAWATER FOR 166 DAYS. MORTALITY IS THE AVERAGE FROM DUPLICATE AQUARIA AND DOES NOT INCLUDE DEATHS WHICH OCCURRED IN THE SPAWNING CHAMBERS.

	Mean measured concentration (µg/ℓ)						
Day	Control	Solvent Control	1.3	4.8	9.6	17.7	34.1
1-40	9	5	6	28	26	34	76
41-80	0	0	0	0	0	0	20
81-120	2	0	0	0	0	30	2
121-166	0	0	0	0	0	2	2
TOTAL	11	5	6	28	26	66[a]	100[a]

[a]Significantly greater than the control.

TABLE 12. GROWTH OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 166 DAYS TO TRIFLURALIN IN FLOWING, NATURAL SEAWATER. MEAN STANDARD LENGTH AND STANDARD DEVIATION ARE GIVEN IN CENTIMETERS AND WERE DETERMINED PHOTOGRAPHICALLY. AVERAGE WEIGHT IS GIVEN IN GRAMS AND WAS DETERMINED IN WATER.

Mean measured concentration (µg/ℓ)	Day 37		Day 94		Day 166	
	Length	Wt.	Length	Wt.	Length	Wt.
Control	1.3±0.3	0.08	3.0±0.2	0.8	3.4±0.3	1.1
Sol. control	1.1±0.3	0.07	2.8±0.3	0.7	3.4±0.3	1.0
1.3	1.3±0.2	0.08	3.0±0.3	0.8	3.5±0.4	1.2
4.8	1.2±0.3	0.08	2.6±0.7	0.7	3.2±0.4	1.0
9.6	1.1±0.2	0.07	2.6±0.4	0.8	2.9±0.4[a]	0.9
17.7	0.9±0.3[a]	0.08	2.2±0.4[a]	0.7	2.7±0.4[a]	0.9
34.1	0.6±0.2[a]	0.01	1.5±0.3[a]	0.2	-[b]	-

[a]Significantly less than the control.
[b]All fish had died.

TABLE 13. NUMBER OF EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) DURING THREE 10-DAY SPAWNING PERIODS; FISH WERE EXPOSED TO TRIFLURALIN IN FLOWING, NATURAL SEAWATER FOR 166 DAYS. FIVE FISH (2 MALES AND 3 FEMALES) WERE PLACED IN A SPAWNING CHAMBER IN DUPLICATE AQUARIA, A AND B.

Day	Mean measured concentration (µg/ℓ)											
	Control		Solvent Control		1.3		4.8		9.6		17.7	
	A	B	A	B	A	B	A	B	A	B	A	B
113 to 122	1,118	559	172	484	232	522	453	410	690	588	62	244
136 to 145	428	940	593	823	115[a]	722[b]	181[b]	264[b]	86	68	39	23
157 to 166	-[c]	489[b]	807	561[b]	459	572[b]	230	618	56	31[b]	-[c]	-[c]
SUB-TOTAL	1,546	1,988	1,572	1,868	806	1,816	864	1,292	832	687	101	267
TOTAL	3,534		3,440[d]		2,622[d]		2,156[d]		1,519[d]		368[d]	

[a]Only one female fish in spawning chamber.
[b]Death(s) occurred in spawning chamber.
[c]No spawning chamber because of lack of surviving fish in appropriate sex ratio.
[d]Siginficantly less than the control.

TABLE 14. NUMBER OF EGGS SPAWNED PER DAY PER FEMALE SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) DURING THREE 10-DAY SPAWNING PERIODS; FISH WERE EXPOSED TO TRIFLURALIN IN FLOWING, NATURAL SEAWATER FOR 166 DAYS. FIVE FISH (2 MALES AND 3 FEMALES) WERE PLACED IN A SPAWNING CHAMBER IN DUPLICATE AQUARIA, A AND B.

Day	Mean measured concentration (µg/ℓ)											
	Control		Solvent control		1.3		4.8		9.6		17.7	
	A	B	A	B	A	B	A	B	A	B	A	B
113-122	37	19	6	16	8	17	15	14	23	20	2	8
136-145	14	31	20	27	12	24	6	9	3	2	1	1
157-166	-[a]	24	27	22	15	23	8	21	2	1	-[a]	-[a]
Mean of duplicate	26	25	18	22	12	21	10	15	9	8	2	4
Mean and S.D. of treatment	25±9		20±8		16±6		12±6[b]		8±10[b]		3±3[b]	

[a]No spawning chamber because of lack of surviving fish in the appropriate sex ratio.
[b]Significantly less than the control.

TABLE 15. HATCHING SUCCESS OF JUVENILES FROM EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO TRIFLURALIN IN FLOWING, NATURAL SEAWATER. PERCENTAGE HATCH AND STANDARD DEVIATION REPRESENT POOLED DATA FROM DUPLICATE AQUARIA DURING THREE 10-DAY SPAWNING PERIODS.

Mean measured concentration (µg/ℓ)	Percentage hatch and S.D.				Number of embryos examined
	1st	2nd	3rd	Mean	
Control	99±1	99±1	97±2	99±1	828
Sol. control	99±2	99±1	98±2	98±2	864
1.3	99±1	84±10	97±3	95±8	671
4.8	99±2	65±28	94±3	90±18	687
9.6	98±3	54[a]	0[b]	91±17	337
17.7	12±10[b]	0[b]	-[c]	12±10	136

[a]Insufficient data for statistical analysis; only one group of embryos.
[b]Significantly less than the control.
[c]No spawning chamber; all surviving fish had been spawned.

TABLE 16. PERCENTAGE MORTALITY, AVERAGE STANDARD LENGTH, AND WEIGHT (DETERMINED IN WATER) OF 28-DAY OLD SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) JUVENILES HATCHED FROM EGGS PRODUCED BY FISH EXPOSED TO TRIFLURALIN FOR 113-122 DAYS.

Mean measured concentration (µg/ℓ)	Number of juv.	Mortality (%)	Length (cm)	Weight (g)
Control	160	14	1.10	0.03
Sol. control	160	15	1.11	0.06
1.3	160	12	1.01	0.05
4.8	160	26	1.00	0.05
9.6	160	44[a]	0.85[b]	0.05
17.7	12	67[a]	1.08[c]	0.10

[a]Significantly greater than the control.
[b]Significantly less than the control.
[c]Not included in statistical analyses; only four surviving fish.

Pentachlorophenol

Exposure to pentachlorophenol significantly reduced survival of parental fish during the 151-day study. All fish exposed to 389 µg/ℓ died within 60 days and mortality of fish exposed to 88 and 195 µg/ℓ was significantly greater than mortality of control fish at the end of the exposure (TABLE 17). A diluter malfunction on day 94 caused low dissolved oxygen concentrations and random mortality. Those deaths were not included in TABLE 17 because they were not considered to be toxicant related.

Pentachlorophenol did not significantly affect growth of surviving parental fish or their fecundity (TABLES A-2--A-4).

Hatching success of juveniles from embryos produced by fish exposed to 195 µg/ℓ during the second spawning period was significantly less than hatching success of control juveniles (TABLE 18).

Mortality of juveniles hatched from embryos produced by fish exposed to 195 µg/ℓ was significantly greater than mortality of control juveniles. Growth of surviving juveniles in all concentrations was not affected, however (TABLE 19).

TABLE 17. CUMULATIVE MORTALITY (%) OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO PENTACHLOROPHENOL IN FLOWING, NATURAL SEAWATER. MORTALITY IS THE AVERAGE FROM DUPLICATE AQUARIA AND DOES NOT INCLUDE DEATHS WHICH OCCURRED IN THE SPAWNING CHAMBERS.

	Mean measured concentration (µg/ℓ)						
Day	Control	Solvent control	18	47	88	195	389
1-30	0	0	0	0	0	0	96
31-60	0	0	0	2	10	10	4
61-90	0	0	0	0	0	0	---
91-120	0	0	0	2	0	12	---
121-151	0	0	0	0	2	6	---
TOTAL	0	0	0	4	12[a]	28[a]	100[a]

[a]Significantly greater than the control.

TABLE 18. HATCHING SUCCESS OF JUVENILES FROM EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO PENTACHLOROPHENOL IN FLOWING, NATURAL SEAWATER. PERCENTAGE HATCH AND STANDARD DEVIATION REPRESENT POOLED DATA FROM DUPLICATE AQUARIA DURING TWO 10-DAY SPAWNING PERIODS.

Mean measured concentration (µg/ℓ)	Percentage hatch and S.D.			Number of embryos examined
	1st	2nd	Mean	
Control	99±2	98±2	98±2	600
Sol. control	100±1	97±3	99±2	732
18	98±3	99±2	98±2	722
47	95±4	99±2	97±3	608
88	91±1	95±6	93±5	350
195	87±10	64±14[a]	77±16	634

[a]Significantly less than the control.

TABLE 19. PERCENTAGE MORTALITY, AVERAGE STANDARD LENGTH, AND WEIGHT (DETERMINED IN WATER) OF 28-DAY OLD SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) JUVENILES WHICH WERE HATCHED FROM EGGS SPAWNED BY FISH EXPOSED TO PENTACHLOROPHENOL FOR 133-142 DAYS IN FLOWING, NATURAL SEAWATER.

Mean measured concentration (µg/ℓ)	Number of juv.	Mortality (%)	Length (cm)	Weight (g)
Control	160	7	1.12	0.04
Sol. control	160	5	1.12	0.03
18	120	3	1.17	0.04
47	120	7	1.07	0.04
88	120	1	1.00	0.03
195	57	28[a]	1.07	0.05

[a]Significantly greater than the control.

BIOACCUMULATION

Chlordane was accumulated by parental fish during the 189-day exposure. Chlordane was also accumulated in eggs/embryos produced by these fish and in 28-day old F_1 juveniles. Concentration factors (based on measured water and tissue concentrations) for parental fish ranged from 13,000-22,000X and were similar to those reported for sheepshead minnows exposed for a much shorter period (Parrish et al., 1976). Concentration factors for juveniles ranged from 6,500-26,800X (TABLES A-5--A-6). No concentration factors are reported for eggs/embryos because the small sample weight caused considerable variability in analytical results (TABLE A-7).

Trifluralin was accumulated by parental fish during the 166-day exposure and by 28-day old F_1 juveniles. Concentration factors (based on measured concentrations) for parental fish ranged from 4,500X-11,500X and concentration factors for juveniles ranged from 1,500X-11,500X (TABLES A-8--A-9). No eggs/embryos were analyzed.

Concentration factors for trifluralin were an order of magnitude greater than those reported for a freshwater fish. Macek et al. (1976) found a maximum concentration factor of 1,333X in fathead minnows. However, tissue concentrations were measured in "eviscerated carcass." Interestingly, in our study the same concentration factor was observed in adult fish that had been exposed for 166 days and in second generation, 28-day old juveniles.

Pentachlorophenol was accumulated by parental fish in the 151-day exposure; pentachlorophenol was also accumulated in eggs/

embryos produced by these fish and in 28-day old F_1 juveniles. Concentration factors for parental fish ranged from 5-27X; for eggs/embryos, 13-26X; and for juveniles, 16-48X (TABLES A-10--A-12). Schimmel et al. (in press) also found that pentachlorophenol was not appreciably accumulated by marine fishes. The maximum concentration factor for longnose killifish was 41X and that for striped mullet, 79X after a 4-day exposure. (The chemical used in the study was sodium pentachlorophenate but tissue concentrations were reported as pentachlorophenol).

TABLE 20. ACCUMULATION OF CHEMICALS BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS). CONCENTRATION FACTORS WERE DERIVED BY DIVIDING MEASURED CONCENTRATIONS IN FISH (WHOLE-BODY, WET-WEIGHT) BY MEASURED CONCENTRATIONS IN TEST WATER.

Chemical	Concentration factor (range)	Exposure duration (days)	Source
Aroclor® 1016	6,900-73,000	28	Hansen et al., 1975
Aroclor® 1254	25,000-55,000	21	Schimmel et al., 1974
Chlordane	8,500-12,300	28	Parrish et al., 1976
	6,500-26,800	28	This study.
	13,000-22,000	189	
Dieldrin	3,500-7,300	4	Parrish et al., 1974
Endrin	3,300-4,800	33	Schimmel et al., 1975
Heptachlor	7,400-21,300	4	Schimmel et al., 1976
Hexachlorobenzene	85-6,700	4	Parrish et al., 1975
Methoxychlor	110-260	140	Parrish et al., 1977
Kepone®	3,600-20,000	36	Hansen et al., 1977
Lindane	340-730	4	Schimmel et al., 1977a
Pentachlorophenol	16-48	28	This study.
	5-27	151	
Toxaphene	3,100-20,600	4	Schimmel et al., 1977b
Trifluralin	1,500-11,500	28	This study.
	4,500-11,500	166	

APPLICATION FACTORS

Application factors were calculated for each of the chemicals based on the results of the acute and chronic toxicity tests (TABLE 21).

TABLE 21. CONCENTRATIONS (µg/ℓ) OF THREE CHEMICALS TOXIC TO SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) IN ACUTE AND CHRONIC TESTS, AND THE RELATIONSHIP OF ACUTE TOXICITY TO CHRONIC TOXICITY.

Chemical	96-hour LC50 (95% confidence limits)	MATC limits	Application factor limits[a]
Chlordane	12.5 (3.4-45.9)	>0.5<0.8	0.04-0.06
Trifluralin	190 (128-282)	>1.3<4.8	0.007-0.025
Pentachlorohenol	442 (308-635)	>47<88	0.11-0.20

[a]Derived by dividing the Maximum Acceptable Toxicant Concentration limits by the 96-hour LC50.

The application factor limits derived for sheepshead minnows and trifluralin were similar to the application factor limits derived for a freshwater fish. We know of no application factors for freshwater fishes and chlordane or pentachlorophenol. However, the similarity of results of life-cycle tests with other chemicals and marine and freshwater fishes support the validity of using sheepshead minnows for conducting life-cycle tests and deriving application factors which can be used to assess the impact of chemicals, particularly pesticides, on freshwater or saltwater environments (TABLE 22).

TABLE 22. COMPARISON OF APPLICATION FACTORS FOR FRESHWATER AND SALTWATER FISHES. APPLICATION FACTOR LIMITS WERE DERIVED BY DIVIDING THE MEASURED WATER CONCENTRATION IN WHICH NO EFFECT WAS OBSERVED AND THE LOWEST MEASURED WATER CONCENTRATION IN WHICH AN EFFECT WAS OBSERVED BY THE 96-HOUR OR INCIPIENT LC50.

Chemical	Species	Habitat	Application factor limits	Source
Carbofuran	*C. variegatus*	Saltwater	0.04-0.06	Parrish et al., 1977
Chlordane	*C. variegatus*	Saltwater	0.04-0.06	This study.
Endrin	*C. variegatus*	Saltwater	0.35-0.91	Hansen and Parrish, 1977
	J. floridae	Freshwater	0.25-0.35	Hermanutz, in press.
Heptachlor	*C. variegatus*	Saltwater	0.09-0.18	Hansen and Parrish, 1977
	P. promelas	Freshwater	0.07-0.15	Macek et al., 1976
Malathion	*C. variegatus*	Saltwater	0.08-0.18	Parrish et al., 1977
	J. floridae	Freshwater	0.02-0.03	Hermanutz, in press.
	L. macrochirus	Freshwater	0.04-0.06	Eaton, 1970
	P. promelas	Freshwater	0.02-0.06	Mount and Stephan, 1967
Methoxychlor	*C. variegatus*	Saltwater	0.24-0.47	Parrish et al., 1977
Pentachlorophenol	*C. variegatus*	Saltwater	0.11-0.20	This study.
Trifluralin	*C. variegatus*	Saltwater	0.007-0.025	This study.
	P. promelas	Freshwater	0.017-0.044	Macek et al., 1976

SUMMARY

1. Chlordane was more acutely toxic to sheepshead minnows than was trifluralin or pentachlorophenol. The estimated 96-hour LC50's, based on measured water concentrations, were 12.5 µg/ℓ, 190 µg/ℓ, and 442 µg/ℓ, respectively.

2. Exposure to chlordane concentrations ≥2.8 µg/ℓ caused significant ($P<0.5$) mortality of parental fish. Exposure to concentrations ≥0.8 µg/ℓ significantly reduced hatch of embryos spawned by parental fish and exposure to concentrations ≥1.7 µg/ℓ caused significant mortality of second generation fish. The estimated MATC of chlordane for sheepshead minnows was >0.5<0.8 µg/ℓ; the AF limits were 0.04-0.06.

3. Exposure to trifluralin concentrations ≥17.7 µg/ℓ caused significant mortality of parental fish. Exposure to concentrations ≥9.6 µg/ℓ significantly reduced growth of parental fish and exposure to concentrations ≥4.8 µg/ℓ significantly reduced fecundity of parental fish. Exposure to concentrations ≥9.6 µg/ℓ significantly reduced hatch of embryos spawned by parental fish, and survival and growth of second generation fish. The estimated MATC of trifluralin for sheepshead minnows was >1.3<4.8 µg/ℓ; the AF limits were 0.007-0.025.

4. Exposure to pentachlorophenol concentrations ≥88 µg/ℓ caused significant mortality of parental fish. Exposure to pentachlorophenol concentrations ≥195 µg/ℓ significantly reduced hatch of embryos spawned by parental fish and survival of second generation fish. The estimated MATC of pentachlorophenol for sheepshead minnows was >47<88 µg/ℓ; the AF limits were 0.11-0.20.

5. The relationship of acute and chronic toxicity for sheepshead minnows exposed to trifluralin (as expressed by an application factor) was similar to that for a freshwater fish exposed to trifluralin.

6. Sheepshead minnows are a suitable saltwater fish for chronic (full life-cycle) toxicity tests, and data from this study and from other studies suggest that application factors derived from tests with pesticides and this fish can be used for freshwater or saltwater fishes.

TABLE 23. SUMMARY OF SIGNIFICANT EFFECTS OF CHLORDANE, TRIFLURALIN, AND PENTACHLOROPHENOL ON SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) DURING CHRONIC (FULL LIFE-CYCLE) EXPOSURES IN FLOWING, NATURAL SEAWATER.

Chemical	Generation	Life stage	Effect	Mean measured concentration (µg/ℓ)
Chlordane	Parental	Adult	Increased mortality	≥2.8
	F_1	Embryo	Decreased hatch	≥0.8
		Juvenile	Increased mortality	≥1.7
Trifluralin	Parental	Adult	Increased mortality	≥17.7
			Decreased growth	≥9.6
			Decreased fecundity	≥4.8
	F_1	Embryo	Decreased hatch	≥9.6
		Juvenile	Increased mortality	≥9.6
			Decreased growth	≥9.6
Pentachlorophenol	Parental	Adult	Increased mortality	≥88
	F_1	Embryo	Decreased hatch	≥195
		Juvenile	Increased mortality	≥195

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APPENDIX

TABLE A-1. GROWTH OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 189 DAYS TO CHLORDANE IN FLOWING, NATURAL SEAWATER. MEAN STANDARD LENGTH AND STANDARD DEVIATION ARE GIVEN IN CENTIMETERS AND WERE DETERMINED PHOTOGRAPHICALLY. AVERAGE WEIGHT IS GIVEN IN GRAMS AND WAS DETERMINED IN WATER.

Mean measured concentration (µg/ℓ)	Day 35		Day 94		Day 189	
	Length (cm)	Wt. (g)	Length (cm)	Wt. (g)	Length (cm)	Wt. (g)
Control	1.3±0.2	0.1	2.9±0.4	0.8	3.6±0.4	1.3
Sol. control	1.3±0.2	0.1	2.9±0.2	0.8	3.7±0.3	1.3
0.5	1.3±0.2	0.1	3.0±0.3	0.8	3.7±0.3	1.4
0.8	1.3±0.2	0.1	2.8±0.4	0.7	3.6±0.4	1.1
1.7	1.3±0.2	0.1	2.8±0.3	0.7	3.5±0.3	1.1
2.8	1.3±0.3	0.1	2.9±0.4	0.8	3.6±0.4	1.6
18.0	-[a]		-		-	

[a]All fish had died.

TABLE A-2. GROWTH OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 151 DAYS TO PENTACHLOROPHENOL IN FLOWING, NATURAL SEAWATER. MEAN STANDARD LENGTH AND STANDARD DEVIATION ARE GIVEN IN CENTIMETERS AND WERE DETERMINE PHOTOGRAPHICALLY. AVERAGE WEIGHT IS GIVEN IN GRAMS AND WAS DETERMINED IN WATER.

Mean measured concentration (µg/ℓ)	Day 34		Day 91		Day 151	
	Length (cm)	Wt. (g)	Length (cm)	Wt. (g)	Length (cm)	Wt. (g)
Control	1.2±0.3	0.06	2.7±0.7	0.7	3.1±0.2	0.8
Sol. control	1.2±0.3	0.08	2.8±0.4	0.7	3.1±0.3	1.0
18	1.3±0.3	0.06	2.7±0.4	0.7	3.1±0.3	0.9
47	1.2±0.3	0.07	2.6±0.3	0.7	3.0±0.2	0.9
88	1.2±0.3	0.06	2.8±0.4	0.7	3.1±0.3	0.9
195	1.3±0.3	0.06	2.6±0.4	0.6	3.1±0.2	0.9
389	1.5±0.1	0.07	-[a]	-	-	-

[a]All fish had died.

TABLE A-3. NUMBER OF EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) DURING TWO 10-DAY SPAWNING PERIODS; FISH WERE EXPOSED TO PENTACHLOROPHENOL IN FLOWING, NATURAL SEAWATER FOR 166 DAYS. FIVE FISH (2 MALES AND 3 FEMALES) WERE PLACED IN A SPAWNING CHAMBER IN DUPLICATE AQUARIA, A AND B.

Day	Mean measured concentration (µg/ℓ)											
	Control		Solvent control		18		47		88		195	
	A	B	A	B	A	B	A	B	A	B	A	B
111 to 120	206	233	378	499	411	348	171	206	114	90[a]	228	531
133 to 142	144	358	242[a]	391	497	482	369	667	643	119[a]	215	327
SUB-TOTAL	350	591	620	890	908	830	540	873	757	209	443	858
TOTAL	941		1,510		1,738		1,413		966		1,301	

[a]Death(s) occurred in spawning chamber.

TABLE A-4. NUMBER OF EGGS SPAWNED PER DAY PER FEMALE SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) DURING TWO 10-DAY SPAWNING PERIODS; FISH WERE EXPOSED TO PENTACHLOROPHENOL IN FLOWING, NATURAL SEAWATER FOR 166 DAYS. FIVE FISH (2 MALES AND 3 FEMALES) WERE PLACED IN A SPAWNING CHAMBER IN DUPLICATE AQUARIA, A AND B.

Day	Mean measured concentration (µg/ℓ)											
	Control		Solvent control		18		47		88		195	
	A	B	A	B	A	B	A	B	A	B	A	B
111-120	8	8	13	17	14	12	6	7	4	4	8	18
133-142	5	12	8	13	17	16	12	22	21	6	7	11
Average of duplicate	6	10	10	15	16	14	9	14	12	5	8	14
Mean and S.D. of treatment	8±3		13±4		15±2		12±7		9±8		11±5	

TABLE A-5. CONCENTRATIONS OF CHLORDANE IN SURVIVING ADULT SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 189 DAYS. MEAN (AND STANDARD DEVIATION) TISSUE RESIDUES ARE WHOLE-BODY, WET-WEIGHT. DUPLICATE ANALYSES OF EACH POOLED SAMPLE (AT LEAST 2 FISH PER SAMPLE) WERE PERFORMED.

Mean measured concentration		Concentration factor	Number of analyses
Water (µg/ℓ)	Tissue (µg/g)		
Control	ND[a]	---	2
Sol. control	ND[a]	---	2
0.5	6.7±3.6	13,400	6
0.8	10.6±3.4	13,250	6
1.7	26.4±15.6	15,529	6
2.8	61.5±33.6	21,964	6

[a]Not detectable; <0.1 µg/g.

TABLE A-6. CONCENTRATIONS OF CHLORDANE IN SURVIVING 28-DAY OLD JUVENILE SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS). THE FISH HATCHED FROM EMBRYOS PRODUCED DURING THE FIRST TWO SPAWNING PERIODS (DAYS 93-102 AND 139-148). MEAN (AND STANDARD DEVIATION) TISSUE RESIDUES ARE WHOLE-BODY, WET-WEIGHT. DUPLICATE ANALYSES OF EACH POOLED SAMPLE FROM DUPLICATE AQUARIA A AND B WERE PERFORMED.

Mean measured concentration				
Water (µg/ℓ)	Tissue (µg/g)		Concentration factor	
	Group 1	Group 2	Group 1	Group 2
Control	-[a]	0.26±0.15	---	---
Sol. control	-[a]	0.30±0.01	---	---
0.5	11±1	4±0	22,000	8,000
0.8	15±3	8±1	18,750	10,000
1.7	33±10	11±0	19,412	6,471
2.8	75±3	31±15	26,786	11,071

[a]No analyses.

TABLE A-7. CONCENTRATIONS OF CHLORDANE IN EGGS/EMBRYOS PRODUCED BY ADULT SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 171-181 DAYS. TISSUE RESIDUES ARE WET-WEIGHT. DUPLICATE ANALYSES OF EACH POOLED SAMPLE FROM DUPLICATE AQUARIA A AND B WERE PERFORMED.

Measured concentration	
Water (µg/ℓ)	Tissue (µg/g)
Control	-[a]
Sol. control	-[a]
0.5	5.5, 5.6, 2.4, 2.8
0.8	83, 84, 31, 32
1.7	46, 47, 413, 403
2.8	23, 19

[a]No analyses.

TABLE A-8. CONCENTRATIONS OF TRIFLURALIN IN SURVIVING ADULT SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 166 DAYS. AVERAGE TISSUE RESIDUES ARE WHOLE-BODY, WET-WEIGHT. DUPLICATE ANALYSES OF A POOLED SAMPLE FROM EACH TREATMENT WERE PERFORMED.

Measured concentration		
Water (µg/ℓ)	Tissue (µg/g)	Concentration factor
Control	0.5	---
Sol. control	2.6	---
1.3	15	11,538
4.8	34	7,093
9.6	46[a]	4,792
17.7	79[a]	4,463

[a]Only one analysis.

TABLE A-9. CONCENTRATIONS OF TRIFLURALIN IN SURVIVING 28-DAY OLD JUVENILE SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS). THE FISH HATCHED FROM EMBRYOS PRODUCED DURING THE FIRST SPAWNING PERIOD (DAYS 113-122). MEAN (AND STANDARD DEVIATION) TISSUE RESIDUES ARE WHOLE-BODY, WET-WEIGHT. DUPLICATE ANALYSES OF EACH POOLED SAMPLE FROM DUPLICATE AQUARIA A AND B WERE PERFORMED.

Mean measured concentration			
Water (µg/ℓ)	Tissue (µg/g)	Concentration factor	Number of analyses
Control	0.9±0.3	---	4
Sol. control	16±11	---	4
1.3	15±14	11,538	4
4.8	14±7	2,917	4
9.6	14±4	1,458	4
17.7	75	4,237	1

TABLE A-10. CONCENTRATIONS OF PENTACHLOROPHENOL IN SURVIVING ADULT SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 151 DAYS. TISSUE RESIDUES ARE WHOLE-BODY, WET-WEIGHT. ANALYSES OF POOLED SAMPLES FROM DUPLICATE AQUARIA A AND B WERE PERFORMED.

Mean measured concentration		Concentration factor	Number of analyses
Water (µg/ℓ)	Tissue (µg/g)		
Control	ND[a]	---	4
Sol. control	0.006	---	4
18	0.48	27	3
47	0.54	11	3
88	0.80	9	4
195	0.97	5	3

[a]Not detectable; <0.005 µg/g.

TABLE A-11. CONCENTRATIONS OF PENTACHLOROPHENOL IN EGGS/EMBRYOS PRODUCED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 133-142 DAYS. TISSUE RESIDUES ARE WET-WEIGHT. ANALYSES OF EACH POOLED SAMPLE WERE PERFORMED.

Measured concentration		Concentration factor
Water (µg/ℓ)	Tissue (µg/g)	
Control	ND[a]	---
Sol. control	ND[a]	---
18	0.24	13
47	0.88	19
88	2.3	26
195	4.3	22

[a]Not detectable; <0.1 µg/g.

TABLE A-12. CONCENTRATIONS OF PENTACHLOROPHENOL IN SURVIVING 28-DAY OLD JUVENILE SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS). THE FISH HATCHED FROM EMBRYOS PRODUCED DURING THE FIRST SPAWNING PERIOD (DAYS 111-120). TISSUE RESIDUES ARE WHOLE-BODY, WET-WEIGHT. ANALYSES OF A POOLED SAMPLE FROM DUPLICATE AQUARIA A AND B WERE PERFORMED

Mean measured concentration		Concentration factor	Number of analyses
Water (µg/ℓ)	Tissue (µg/g)		
Control	ND[a]	---	4
Sol. control	ND[a]	---	4
18	0.86	48	3
47	1.6	34	3
88	1.4	16	4
195	7.4	38	3

[a]Not detectable; <0.01 µg/g.